

MANAGING CHRONIC PAIN WITH ENCAPSULATED CELL IMPLANTS RELEASING CATECHOLAMINES AND ENDOGENOUS OPIOIDS

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Materials and methods
 - 3.1. Chromaffin cell isolation and culture
 - 3.2. Device fabrication and encapsulation procedure
 - 3.3. Neurochemical assays
 - 3.4. Implantation and retrieval
 - 3.5. Histology
 - 3.6. Data analysis
4. Results
 - 4.1. Chromaffin cell isolation and culture
 - 4.2. Neurochemical analyses
 - 4.3. Implant retrieval
 - 4.4. Histology
5. Discussion
 - 5.1. Observations from the present study
 - 5.2. Preclinical studies using unencapsulated chromaffin cells
 - 5.3. Clinical trials using unencapsulated chromaffin cells
 - 5.4. Clinical trials using encapsulated chromaffin cells
 - 5.5. Additional comments and conclusion
6. Acknowledgments
7. References

1. ABSTRACT

Spinal injections (intrathecal) of norepinephrine and/or opioid agonists are antinociceptive and when administered together may act in synergy. Spinal implants of adrenal chromaffin cells are an effective method for sustained delivery of the analgesic substances norepinephrine and enkephalin to the central nervous system (CNS). One method of packaging and implanting cell-loaded devices into the intrathecal space of recipients is by encapsulating the cell suspensions in a polymer membrane prior to implantation. Cells/tissue packaged within an encapsulating membrane obviate the need for immunosuppressive therapies in transplant recipients. In addition, device output can be quantified prior to implantation, and following the removal of the spinal implant. The ability to retrieve the devices with the present tubular configuration also confers an additional margin of safety over unencapsulated chromaffin cell implants.

This paper reviews the research and clinical observations of cellular transplants containing adrenal chromaffin cells for relieving chronic pain. Encapsulated cell technology is discussed with an emphasis on our experiences developing pain-modulating clinical devices. The human-sized prototype devices were loaded with enzymatically isolated bovine chromaffin cells and maintained *in vitro* for 7 - 8 days in serum-free media. Two

days prior to implantation, each device was assayed by static incubation to measure catecholamine and met-enkephalin output, and qualified devices (n = 6) were implanted into the sheep subarachnoid space for 6 weeks. Following a 6 week in life period, the retrieval forces of prototype devices were measured during removal from the subarachnoid space. Static incubation of the devices immediately following retrieval and after a 24 hour re-incubation period were used to quantify norepinephrine and met-enkephalin secretion profiles. This study demonstrated the safety, retrievability and maintenance of pharmacologically active encapsulated chromaffin cell-loaded devices with human implant dimensions.

2. INTRODUCTION

Transplanted primary adrenal chromaffin cells have been used for the sustained delivery of therapeutic molecules for indications such as Parkinson's disease, depression, traumatic brain injury, stroke and pain (reviewed in 1-5). Adrenal chromaffin cells, isolated from a number of different species, have demonstrated the ability to produce catecholamines (6), opioid peptides (6-9), as well as neuropeptides such as neuropeptide Y, (10) neurotensin, and somatostatin (11). These substances induce postsynaptic inhibition of afferent nociceptive impulses

Clinical Prototype Pain Relieving Devices

within the dorsal horn of the spinal cord. Yaksh *et al.* (12), demonstrated that intrathecally administered opiates, alpha-adrenergic agonists and baclofen produced a long-lasting analgesia which was antagonized by naloxone. Co-administration of opiates and alpha-adrenergic agonists exhibited a synergistic effect in pain reduction by activating opiate and alpha-adrenergic receptor systems in the spinal cord. The analgesic effects obtained in pain models by selectively activating these receptor systems was the basis for the advancements by Sagen *et al.* (13-17) who utilized adrenal medullary cellular transplants to induce antinociception in pre-clinical pain models. The encouraging pre-clinical results with adrenal medullary tissue and chromaffin cell preparations in models of both acute and chronic pain led to small clinical trials in end-stage cancer patients with intractable chronic pain unresponsive to conventional aggressive oral therapy (18-21).

Chronic pain is a prevalent worldwide problem with significant physical, psychological, and social impacts. Chronic pain is usually defined as either lasting a month or more beyond the typical recovery period for a given illness/injury or extending for years as a result of a chronic or debilitating disease state. Chronic pain may be a primary condition generally related to musculoskeletal trauma, surgical complications, fibromyalgia, and cancer pain (22). In addition, chronic pain can be a secondary problem in persons who already have a disability such as spinal cord injury, multiple sclerosis, cerebral palsy, phantom limb pain, or postpolio syndrome. Chronic pain affects nearly 90 million individuals in the United States with an estimated annual cost of 90 billion dollars as a result of direct medical costs, sick time, and reduced productivity (22). There are also immeasurable costs to quality of life and family structure.

Since chronic pain cannot be cured but only managed, it is an ongoing source of frustration for the health care professional. For example, the World Health Organization (WHO) three-step (ladder) analgesic approach outlines a "standardized" approach to the treatment of cancer pain utilizing non-opioid analgesics, adjuvants, and lastly, opioids (23). While systemic opioid therapy is the gold standard of pharmacological care, its users suffer from: (1) side-effects including sedation, nausea, dysphoria, and constipation, necessitating additional and frequent medications which produce additional side-effects; (2) pharmacokinetic profiles from oral delivery that produce overlapping episodes of pain resistance and impaired quality of life, and; (3) tolerance to opioid pain control (24).

One means of overcoming many of these issues is to use continuous delivery of opioids intrathecally via surgically-implanted pumps. While this effectively reduces chronic pain and potentially mitigates the side effect profile of opioids, continuous delivery pumps are more costly, more complex, and are associated with increased risks compared with oral delivery (24). Like any surgical procedure, implantation and removal of the device requires hospitalization, and continued maintenance and monitoring

of the implanted device. To maintain long-term delivery, repeated access to the device is needed resulting in additional expense and increased risk of bacterial contamination that may be life-threatening to these already compromised patients. While these systems represent a clear improvement over systemic delivery, they also highlight the need for a continuous means of delivering effective pain medications without the obstacles presented by traditional mechanical devices.

Delivering antinociceptive molecules is possible using cells as natural biological mini-pumps to deliver efficacious compounds to the CNS. Specifically, the transplantation of adrenal chromaffin tissue into the subarachnoid space has emerged as a potential new pain therapy for chronic cancer and benign pain states (13-21, 25-32).

In principle, cell transplantation enables long-term pain control at the spinal cord level without the need for repeated interventions associated with conventional intrathecal drug delivery. One iteration of cell-based therapy proposes to use xenogeneic chromaffin cells that are encased within a selectively permeable polymeric membrane; known as immunoisolation. Immunologically isolated (encapsulated) bovine chromaffin cells implanted in the subarachnoid space have provided antinociceptive effects similar to those obtained with unencapsulated cells demonstrating that the therapeutic molecules of interest are able to traverse the encapsulating membrane (33). Immunoisolation is based on the observation that xenogeneic cells can be protected from host rejection by encapsulating, or surrounding them within an immunoisolatory, semipermeable membrane (figure 1). Single cells or small clusters of cells can be enclosed within a selective, semipermeable membrane barrier which admits oxygen and required nutrients and releases bioactive cell secretions, but restricts passage of larger cytotoxic agents from the host immune defense system. The selective membrane eliminates the need for chronic immunosuppression of the host and allows the implanted cells to be obtained from non-human sources, thus avoiding the constraints associated with cell sourcing, which have limited the clinical application of unencapsulated cell transplantation. Aebischer *et al.* (34) initially demonstrated encapsulated cell viability of a xenograft for at least 12 weeks in a parenchymal model of neural transplantation without suppressing the host's immune system.

Several types of cell immunoisolation devices have been developed, but they all are based on the premise that the membrane barrier isolates cells from the host immune system while permitting the bi-directional flux of molecules across the membrane (figure 1). Extra-vascular chambers were initially developed such that blood flowed through a shunt comprising a tubular membrane with cells in an external compartment. This type of configuration utilized dialysis membranes within an extra-corporeal artificial kidney device (35). A second configuration is comprised of microspheres in which cells are surrounded with a thin, spherical, semi-permeable polymer film in the 0.05 – 0.5 mm diameter (36). Alginate:poly-L-lysine complexed

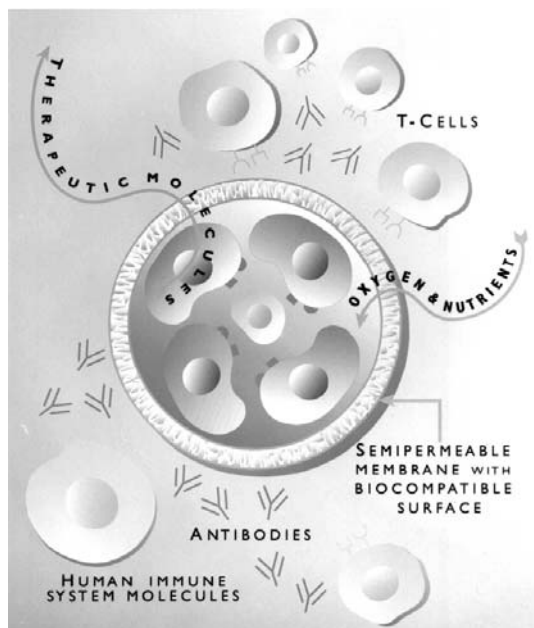


Figure 1. The concept of immunoisolation consists of living cells in supportive matrices encapsulated within a semipermeable polymer membrane. Molecules such as oxygen, glucose, other nutrients, and therapeutic substances like norepinephrine and met-enkephalin have access through the selectively permeable polymer membrane, but larger host immune elements are inhibited from making contact with the encapsulated cells, hence the cells are isolated from the immune system.

polyelectrolyte microcapsules containing bovine adrenal chromaffin cells have been utilized in a unilateral 6-OHDA hemiparkinsonian rat model (37). Striatal implants of the chromaffin cell encased microcapsules reduced dopamine receptor agonist rotational asymmetry, confirming the presence of a sustained release of dopamine or dopamine receptor agonists. The small size, thin wall, and spherical shape is structurally optimal for diffusion, cell viability, and release kinetics. However, in certain methods of thermoplastic-derived processing to form the microcapsule membrane around cellular clusters, cells can be exposed to organic solvents (36, 38). Other types of microcapsules, e.g., the polyelectrolytes, although nontoxic during processing, are generally mechanically and chemically fragile, as well as difficult to retrieve (36). The third configuration is comprised of rod, disc, or sheath macrocapsules (35). Macroencapsulation involves filling a hollow, usually cylindrical, selectively permeable membrane with cells, generally suspended in a matrix, and then sealing the ends to form a capsule (reviewed in 39-41). Polymers used for macroencapsulation are biodegradable, with a thicker wall than that found in microencapsulation. Macroencapsulation is generally achieved by filling preformed thermoplastic hollow fibers with a cell suspension. The hollow fiber is formed by pumping a solution of polymer in a water miscible solvent through a nozzle concurrently with an aqueous solution. The polymer solution is pumped through an outer annular region of the nozzle, while the aqueous solution is pumped through a

central bore. Upon contact with the water, the polymer precipitates and forms a cylindrical hollow fiber with a permselective inner membrane or "skin". Further precipitation of the polymer occurs as the water moves through the polymer wall, forcing the organic solvent out and forming a trabecular wall structure. The hollow fiber is collected in a large aqueous water bath, where complete precipitation of the polymer and dissolution of the organic solvent occurs. The ends of the hollow fiber are then sealed to form macrocapsules. This final step is not a trivial one, since reliably sealing the ends of capsules can be extremely difficult, and provides the barrier paramount for successful immunoisolation. While thicker wall and larger implant diameters can enhance long-term implant stability, these features may also impair diffusion, compromise the viability of the tissue, and slow the release kinetics of desired factors. Cell-loaded macrocapsules similar to those described in the present studies have exhibited the ability to be retrieved (42) from the recipient and may be replaced if necessary (figure 2).

The present report provides an overview of the pre-clinical and clinical use of chromaffin cells to treat chronic pain and also provides some new data describing the development and safety testing of clinical pain-modulating prototype devices in a large animal model. These human-sized prototype devices were loaded with enzymatically isolated bovine chromaffin cells and qualified devices (n = 6) were implanted into the sheep subarachnoid space for 6 weeks. Following a 6 week in life period, the retrieval forces of prototype devices were measured during removal from the subarachnoid space. Static incubation of the devices immediately following retrieval and after a 24 hour re-incubation period was used to quantify norepinephrine and met-enkephalin secretion profiles. This study demonstrates the safety, retrievability, and maintenance of viable encapsulated bovine chromaffin cell-loaded devices exhibiting human implant dimensions.

3. MATERIALS AND METHODS

3.1. Chromaffin cell isolation and culture

The adrenal chromaffin cell preparations were isolated as described previously (43). Adrenal glands were isolated and excised en-bloc and maintained in sterile Hanks Balanced Salt Solution (HBSS, Gibco / InVitrogen, Grand Island, NY). Unessential connective, adipose, and vasculature tissues were trimmed away and each gland was connected to a 22-gauge catheter to infuse and perfuse the glands with HBSS, followed by approximately 50 mL of a warm digestion solution (0.1% (w/v) Collagenase in HBSS). Each gland was immersed in digestion solution and maintained at 37 °C for 45 min. The inner medullary region of each gland was removed with a sterile spatula and the collected digested tissue was triturated to generate a cell suspension. The cell suspension was passed through a 200 µm mesh screen, centrifuged for 3 min at 70 x g, the supernatant discarded, the pellet re-suspended in serum-free media and the process was repeated 3 times with slightly increasing centrifugation (70, 80, 100 x g). The final cell pellet was re-suspended in antibiotic-free chinese hamster ovary serum-free media (CHO-S-SFM-II(H), Gibco

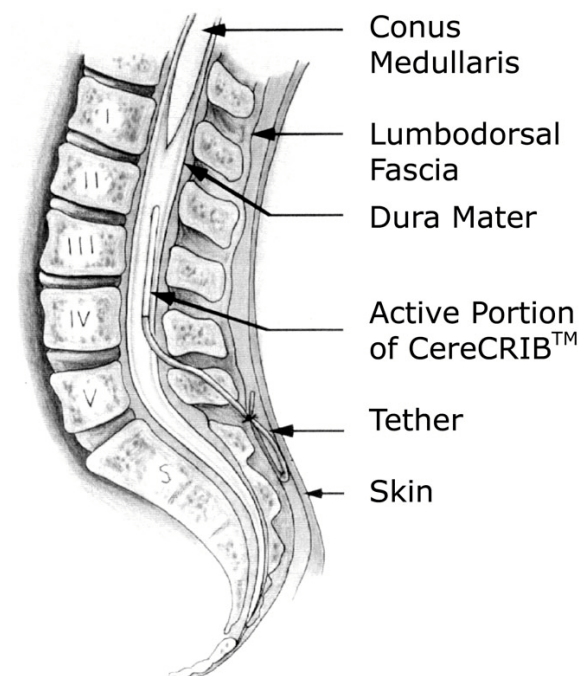


Figure 2. The clinical prototype devices (i.e., CereCRIB™: Cellular Replacement by Immunolatory Biocapsule) may be used to treat chronic pain by implanting encapsulated adrenal chromaffin cells, which release the naturally existing analgesic substances norepinephrine and met-enkephalin, into the spinal subarachnoid space. An implant into the lumbar region (L4/5 to L2) should safely miss the spinal cord at the L1 level and may provide more effective pain relief than traditional approaches.

/InVitrogen) and plated onto non-tissue culture treated T150 cm² flasks and incubated for 4 – 6 days at 37 °C in a humidified 5% CO₂ environment. Microbial bioburden was assessed by inoculating approximately 5% of the cell isolation into trypticase soy broth and fluid thioglycollate medium.

3.2. Device fabrication and encapsulation procedure

Ultrafiltration grade hollow fiber membranes were prepared with a phase inversion technique using poly (acrylonitrile-co-vinyl chloride, Union Carbide, Somerset, NJ) dissolved in dimethyl sulfoxide (Fisher Scientific, Pittsburgh, PA). Hollow fibers were impregnated and stored in glycerin to preserve the membrane porosity and permeability characteristics. These hollow fibers, with dimensions of approximately 950 μm outer diameter and 140 μm wall thickness, exhibited a dense selectively permeable smooth membrane on both the luminal and external surfaces with supporting trabecular macrovoids comprising the wall structure (44). The hollow fibers exhibited a hydraulic permeability of 16 mL/min/m²/mmHg and a rejection coefficient of 85% for bovine serum albumin (BSA) (44). Prototype clinical devices were fabricated by adhering a 5 cm long segment of the hollow fiber membrane with a light-cured acrylate to a pre-formed acrylic hub (septal fixture) mounted to a 25-gauge needle attached to the top of a 15 mL centrifuge tube. The distal extremity of each segment was later sealed with the light-

cured acrylate to form a smooth, rounded extremity. Mounted devices were qualified by examining their ability to resist the passage of air within defined acceptance criteria and further evaluated for hydraulic permeability and rejection coefficients for BSA. The devices were sterilized by exposure to ethylene oxide and stored until loaded with chromaffin cell preparations.

Qualified, sterile prototype encapsulation devices were de-glycerinized in 70% filter sterilized ethanol and calcium-magnesium free HBSS. The adrenal chromaffin cell preparations were collected from the non-tissue culture treated T150 cm² flasks with the aid of a washing stream from the conditioned media in each flask. The cell preparations were pelleted, re-suspended to a final concentration of 1.5% sodium alginate (Ultrapure, Protan, Norway) and 2.2 x 10⁶ cells in a 22 μl volume were loaded via a Hamilton syringe into each device through the septal fixture (port). The septal fixture was snapped off and a silicone tether mounted to a titanium insert was adhered to the proximal end of the prototype device with the light-cured acrylate. Individual cell-loaded devices were evaluated microscopically for implant acceptance criteria, transferred to prototype shipping devices containing the CHO-S-SFMII(H) media, and maintained in a 37 °C incubator for at least 7 days prior to implantation.

3.3. Neurochemical assays

One day prior to implantation (pre-), the catecholamine and met-enkephalin output from each device (n = 10) was quantified as previously described by HPLC and radioimmunoassay (RIA), respectively (44). The prototype devices were removed from their shipping devices, placed in a sterile testing chamber, and washed 2x in 2 ml HBSS containing 2 μM ascorbate and 10 mM HEPES for 15 min each. A third 2 ml HBSS solution was maintained with the submerged devices for 30 min to be utilized for calculating the basal output from the prototype pain-modulating devices. After removal of the basal media, a 2 ml solution of the HEPES-buffered HBSS (with ascorbate) containing 20 μM nicotine tartrate was placed over the devices in the testing chamber to induce an evoked release of neurochemicals from the encapsulated chromaffin cell preparations. The basal and evoked samples were removed from the testing chamber and half (1 ml) placed in a tube containing perchloric acid and stored at –80 °C until analyzed for catecholamines. The other half (1 ml) was placed in a second tube and stored at –80 °C until analyzed for met-enkephalin. The devices were placed back into the prototype shipping devices. Six of the devices that met neurochemical release qualifications were assigned implant status and held until transport to the animal facility for implantation into the subarachnoid space.

Following explantation (post-), an immediate basal and nicotine-evoked catecholamine and met-enkephalin sample from each device was quantified from 2 ml HEPES-buffered HBSS with ascorbate as described above. Thereafter, the devices were placed back into their original prototype shipping devices containing the CHO-II-SFM(H) and maintained in the 37 °C incubator overnight. At 24 hours after device retrieval from the sheep subarachnoid

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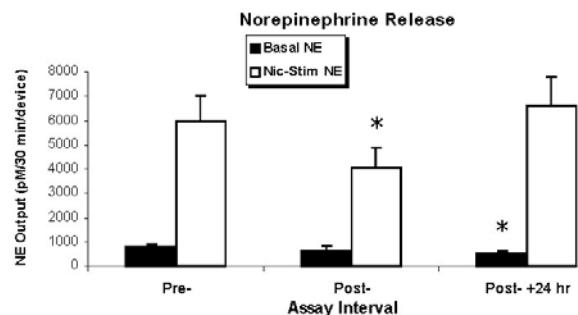


Figure 3. The norepinephrine output from the prototype devices was measured under basal and nicotine-stimulated (20 μ M) conditions prior to implantation (pre-), at explant (post-), and 24 hr after a re-incubation period (post- + 24 hr). Data are presented as mean \pm SD (n = 6). A statistically significant decrease (*) in norepinephrine output was observed under basal conditions at the post- + 24 hr culture period as compared to the pre-implant values ($p < 0.01$). In addition, a significant drop in nicotine-stimulated output was observed in devices immediately retrieved (post-) as compared to the pre-implant output (pre-).

space (post- 24), a basal and nicotine-evoked sample was again collected as described for the pre- and post- samples previously. Following the post- 24 hr assay, each cell-loaded device was fixed by immersion in 4% paraformaldehyde.

3.4. Implantation and retrieval

Sheep (56 ± 12 kg) were sedated with intravenous 10 mg/kg sodium pentobarbital, followed by endotracheal anesthesia with 1 – 2% halothane. All animals received pre- and post-operative antibiotics (20 mg / kg sodium cefazolin). Animals were placed in the prone position on the operative table with their heads tilted at 25 – 30°. A 4 – 8 cm parasagittal lumbar incision was made, the tissue was dissected to the dorsal fascia, and the subarachnoid space was punctured for a spinal tap with a 22-G Tuohy needle between L4 and L5. The CSF was examined for quality assurance (cell counts, microbiology), and 1 ml was processed for pre- (and post- during explant) neurochemistry for norepinephrine and met-enkephalin as described above. A guide wire was introduced through the lumen of the Tuohy needle. The Tuohy needle was removed and a dilator was introduced over the guide wire to expand the fascia, paraspinous muscle, and the ligamentum flavum. A 4-French cannula was advanced to the subarachnoid space and further advanced 7 – 8 cm into the space. The guide wire and dilator were removed and a clinical pain-modulating prototype device was prepared to enter the cannula. Immediately before the prototype devices were implanted, a 1 ml sample of cerebro-spinal fluid (CSF) was collected for analysis of norepinephrine, met-enkephalin, glucose, cell counts, total protein, and bacteriology. For the implantation procedure, a stainless steel pusher was inserted into the flexible silicon tether, the prototype device carefully inserted into the cannula, and advanced until the membrane portion of the device (5 cm) was within the fluid-filled subarachnoid space. The cannula was removed followed by the pusher. The silicone tether extending out of the implantation track was sutured to the

fascia and covered with a two layer closure. The animals were allowed to recover and monitored at 2 hr and 24 hr for pain management and neurologic complications.

At 6 weeks following the implantation procedure, the sheep were anesthetized as described above. The silicon tether of each device was localized and a spinal needle was utilized to collect 1 ml of CSF for analysis of norepinephrine, met-enkephalin, glucose, cell counts, total protein, and bacteriology. Thereafter, a strain gauge (Instron Corp., Canton, MA) was utilized to measure the maximum force required to retrieve the prototype devices from the subarchnoid space. The devices were placed in their prototype shipping device and assayed for neurochemical output at explant (post) and post- + 24 hr. CSF samples were collected to measure norepinephrine and met-enkephalin content as described above. Thereafter, the animals were sacrificed with an overdose of pentobarbital.

3.5. Histology

Once the devices were fixed in the 4% paraformaldehyde, they were washed in PBS, dehydrated to 100% ethanol and embedded in glycol methacrylate (Historesin, Reichert-Jung). Sections 2 – 4 μ m in thickness and approximately 1 cm in length were mounted on glass slides and stained with either cresyl violet or hematoxylin and eosin (H and E).

3.6. Data analysis

Data are presented in the text and all figures as the mean \pm standard deviation. Statistical analyses were conducted for the neurochemical output with a paired student t-test. Significance was established at $p < 0.05$.

4. RESULTS

4.1. Chromaffin cell isolation and culture

The chromaffin cell isolation and culture process was carried out in a class 10,000 facility where all buffers and tissue culture media were free of antibiotics. Bioburden assessment of chromaffin cell lots demonstrated one positive case out of 23 isolation procedures (4.35%). The isolation and culture procedure generated a range of 2.5 to 4.0×10^7 cells per gland of encapsulation quality material. The viability, as assessed with trypan blue exclusion, was $94 \pm 3\%$ at the time of harvest. The isolated cell preparations generally organized themselves into clusters ranging from 30 – 100 μ m in diameter.

4.2. Neurochemical analyses

The six devices implanted into the sheep subarachnoid space were qualified for basal and nicotine-stimulated norepinephrine (NE) release prior to implantation (pre-), immediately following retrieval after a six week implant duration (post-), and following a 24 hr culture period *in vitro* (post- + 24 hr; figure 3). The basal output of NE immediately following retrieval (649 ± 164 pM/30 min/device) was not significantly different than the pre-implant output (757 ± 151 pM/30 min/device). A statistically significant difference ($p < 0.01$) was observed between the pre-implant basal output and following the post- + 24 hr culture period (Figure 3). In addition, a significant

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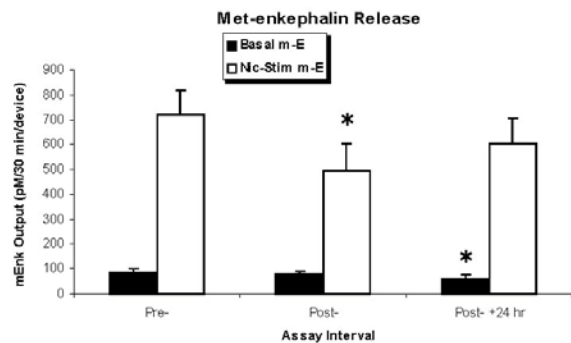


Figure 4. The met-enkephalin output from the prototype devices was measured under basal and nicotine-stimulated (20 μ M) conditions prior to implantation (pre-), at explant (post-), and 24 hr after a re-incubation period (post- + 24 hr). Data are presented as mean \pm SD (n = 6). A statistically significant decrease (*) in met-enkephalin output was observed under basal conditions at the post- + 24 hr culture period as compared to the pre-implant values ($p < 0.01$). A significant difference was also observed between the post- and post- + 24 hour sample times ($p < 0.01$). As was observed with norepinephrine, a significant drop in nicotine-stimulated output was observed in devices immediately retrieved (post-) as compared to the pre-implant output (pre-).

drop in nicotine-stimulated output was observed in devices immediately retrieved (post-; 4035 ± 863) as compared to the pre-implant output (5971 ± 1027). Interestingly, following a post- 24 hr culture period (figure 3), no differences in nicotine-stimulated output were observed between the pre- implant output and the post- + 24 hr (6584 ± 1205).

As described for the NE output, the devices were also characterized for the output of met-enkephalin prior to implantation (pre-), immediately following retrieval after a six week implant duration (post-), and following a 24 hr culture period *in vitro* (post- + 24 hr; figure 4). The basal output of met-enkephalin immediately following retrieval (80.5 ± 8.9 pM/30 min/device) was not significantly different than the pre- implant output (87.2 ± 12.4 pM/30 min/device). A statistically significant difference ($p < 0.01$) was observed between the pre-implant basal output and following the post- + 24 hr culture period (63.8 ± 11.5 ; Figure 4). A significant difference was also observed between the post- and post- + 24 hour sample times ($p < 0.01$). In addition, a significant drop in nicotine-stimulated output was observed in devices immediately retrieved (post-; 496.8 ± 111.9) as compared to the pre-implant output (723.2 ± 94.3). Similar to the NE observations, following a post- 24 hr culture period (figure 4), no differences in nicotine-stimulated met-enkephalin output were observed between the pre- implant output and the post- + 24 hr (607.7 ± 98.5).

Immediately before implantation of the prototype devices, and at the time of device retrieval (at 6 weeks), the 1 ml of CSF was examined for norepinephrine, met-enkephalin, glucose, cell counts, total protein, and bacteriology. No differences were observed for glucose,

cell counts, total protein, or bacteriology. Table 1 provides a summary of the norepinephrine and met-enkephalin levels in the CSF prior to implantation (pre-implant) and at device retrieval (explant). Prior to implantation, the CSF exhibited a NE concentration of 1.22 ± 0.4 pM/ml. At the time of device retrieval, a statistically significant increase in CSF NE was observed (2.73 ± 0.79 pM/ml; $p < 0.01$). Additionally, a significant increase in CSF met-enkephalin was observed between pre-implant (26.9 ± 3.4 pM/ml) and immediately prior to device retrieval (33.1 ± 4.6 ; $p < 0.01$).

4.3. Implant retrieval

Table 2 provides a summary of the clinical observations during recovery of the sheep that received the prototype devices implanted into the subarachnoid spinal space at 2 and 24 hr following the implant procedures. A slight to moderate instability in the hindlimbs was observed in 5 of the 6 recipients at the 2 hr observation period. Two of the 5 animals favored their right hindlimb, and one of these animals continued to exhibit a slight favoring of the right hindlimb at the 24 hr observation point. Five of the 6 recipients demonstrated no additional complications after the 2 hr observation period and one of the animals exhibited no transient effect at any time (table 2). A strain gauge was utilized to measure the maximum pull force required to remove the prototype devices from the subarachnoid space at explant. The maximum pull force required to retrieve each device is outlined in Table 1. A maximum of 8 g of force was required to extract one of the devices; 2 of the devices required a pull force of 7 g; 1 at 5g; and the final 2 devices needed 2 g pull force for retrieval. Some adherent host tissue was noted on 1 of the 6 retrieved devices at explant (table 2).

4.4. Histology

Microscopic analysis of each device demonstrated an abundance of viable cell aggregates as assessed with H and E (figure 5). The contracted alginate matrix, an artifact of the dehydration process to prepare the devices for polymer embedding, can be observed surrounding the viable cell aggregates. The devices demonstrated a relatively consistent pattern of cell aggregate dispersion along the length of the device, although a 20 - 30% increase in the packing density could be observed in the distal-most region (last 1 cm) of the 5 cm prototype devices.

5. DISCUSSION

5.1. Observations from the present study

The present study demonstrated the safety and six week survival of a discordant xenotransplant of encapsulated bovine cells in a sheep spinal model utilizing a clinical prototype pain-relieving device secreting the antinociceptive compounds norepinephrine (NE) and met-enkephalin. The minimally-invasive implantation procedure was well tolerated and 5 of the 6 animals exhibited slight to moderate transient clinical sequelae of hindlimb instability when examined at 2 and 24 hrs with no additional complications noted thereafter. After the six week implant duration, the devices were easily retrieved, with a maximal pull force of 8 g measured. Neurochemical

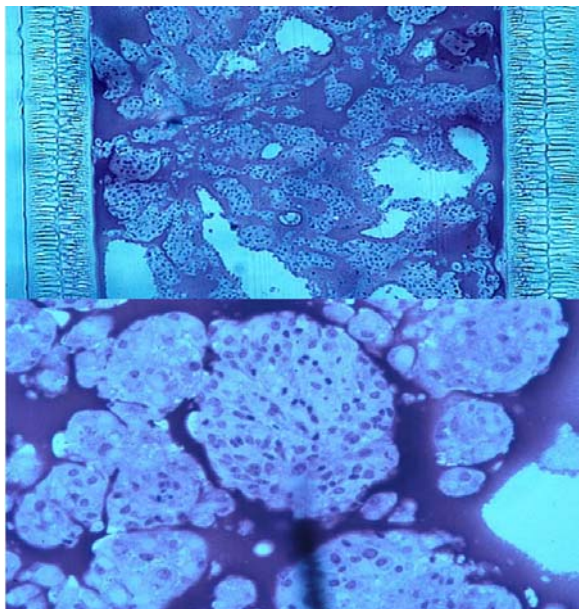


Figure 5. Longitudinal H and E-stained sections of the clinical prototype chromaffin cell-loaded devices after the post- + 24 hr assay neurochemical samples were collected. The contracted alginate matrix, an artifact of the dehydration process to prepare the devices for glycol methacrylate embedding, can be observed surrounding the viable chromaffin cell aggregates. The devices demonstrated a relatively consistent pattern of cell aggregate distribution although some packing (20-30% increase) could be observed in the distal region (~1 cm).

release profiles of norepinephrine and met-enkephalin from each device were measured prior to implantation (pre-), immediately following retrieval after a six week implant duration (post-), and following a 24 hr culture period *in vitro* (post- + 24 hr). Data from the present study demonstrated the maintenance of viability with sustained secretion profiles of NE and met-enkephalin.

The present study extends previous reports (42) with several new and distinguishing points that deserve mention. The present study utilized an FDA-approved cell processing facility to procure and maintain the chromaffin cell aggregates. This study also utilized a clinical prototype device and implantation system supporting an IND submission. The clinical observations at recovery were carefully monitored and described. Although transient clinical sequelae of hindlimb instability was observed, the spinal cord in sheep anatomy extends to the L4 – L5 level, and the implant is placed over the spinal cord. Thus, the neurologic complications would not be expected in humans where the spinal cord ends at the L1 level. For the present study, the force required to retrieve each device was quantified, and the neurochemical output from each device was meticulously monitored. Lastly, implant-related infections are always of concern. Each device is carefully monitored for sterility prior to and during implantation. If a bioburden became positive following the implant procedure, the technology provides a readily available system for easy access and removal. The glucose, cell

counts, total protein and bacteriology results from the present study were all in a range to support the observation of implant sterility.

The present data also confirm two major advantages of polymer encapsulation over traditional, unencapsulated implants. First, encapsulation allows the use of discordant, even xenogeneic tissue, to be used for transplantation. The clinical prototype devices described here enabled bovine chromaffin cells to survive and function following transplantation into the central nervous system (CNS) of a xenogeneic host. While the blood-brain barrier in the CNS confers a degree of protection against invasion by immune components, this privilege is not absolute and discordant xenogeneic tissues are normally rapidly rejected following transplantations. Bovine chromaffin cells potentially provide a virtually unlimited source of transplantable tissues that can be confirmed to be viral and pathogen free. As such, this encapsulation system could potentially treat the majority of patients suffering from chronic pain in a safe, reliable, and reversible manner. A second major advantage of this system is that it avoids the use of chronic, systemic immunosuppression in an already compromised population of patients. But, even if drug regimens were developed to support xenograft transplantation, the available immunosuppressive agents have side effects that severely limit their use. This is especially true in children where the risk of infection and malignancy (45, 46), and stunting of normal growth and development, preclude immunosuppression in the absence of life-threatening organ failure or other serious complications of diabetes (47). Many currently used immunosuppressive drugs are detrimental to the survival and function of the transplanted cells (48, 49). To date, polymer encapsulation represents the only method of ensuring long-term xenogeneic cell survival in both pre-clinical and clinical systems (33, 34, 43, 50-56).

5.2. Preclinical studies using unencapsulated chromaffin cells

Because chromaffin cells secrete numerous antinociceptive compounds, investigators have implanted adrenal medullary tissue grafts into the central neuraxis of rats. Using several standard pre-clinical analgesic drug screening tests, including the hot plate, paw flinch, and tail flick tests, Sagen and colleagues have demonstrated efficacy in rats receiving adrenal medullary implants onto the spinal cord (13-17, 25-28). Importantly, these effects were dependent on systemic nicotine administration to stimulate catecholamine and enkephalin/endorphin release from the chromaffin cells. Neurochemical evaluations of the CSF revealed elevated catecholamine and met-enkephalin concentrations suggesting a causal relationship between these compounds and behavioral efficacy (17). These effects were also partially blocked by co-administration of the opioid antagonist naloxone or the adrenergic antagonist phentolamine. Similar tests of adrenal chromaffin cell implants conducted in chronic pain models demonstrated efficacy in the inflammatory polyarthritis model and in the sciatic nerve chronic constriction injury (CCI) model (16, 25-27). Hama and Sagen reported that within one week of implantation chromaffin cell

Clinical Prototype Pain Relieving Devices

Table 1. The Values of Norepinephrine and Met-enkephalin in Sheep CSF

Norepinephrine (pMol/ml)		Met-enkephalin (pMol/ml)	
Pre-implant	Explant	Pre-implant	Explant
1.22 ± 0.4	2.73 ± 0.79	26.9 ± 3.4	33.1 ± 4.6

CSF Levels of Norepinephrine and Met-enkephalin prior to implantation (pre-implant) and following the removal (explant) of the chromaffin cell-loaded clinical prototypes. Data are presented as mean ± SD (n = 6). A statistically significant increase in both norepinephrine and met-enkephalin was observed in the CSF of sheep at explant as compared to the pre-implant values (p < 0.01)

Table 2. Clinical Observations During Recovery of the Sheep Following the Implantation Procedure

Recovery Observations	Pull Force	Retrieval Comments
Instability in hindlimbs at 2 hr check; no further complications noted	5 g	Removed easily and intact
Instability in hindlimbs at 2 hr check; no further complications noted	2 g	Removed easily and intact
Favored right hindlimb at 24 hr check; no further complications noted	7 g	Removed easily and intact
Instability in hindlimbs at 2 hr check; no further complications noted	8 g	Removed easily and intact
Favored right hindlimb at 2 hr check; no further complications noted	7 g	Removed easily and intact; some adherent host tissue noted
No instability noted at 2 hr check; no complications noted	2 g	Removed easily and intact

Clinical observations of the sheep during their recovery at 2 and 24 hr following the implantation of the chromaffin cell-loaded clinical prototypes in the subarachnoid spinal space. A slight to moderate instability in the hindlimbs was observed in 5 of the 6 recipients at the 2 hr observation period. The maximum pull force to explant the prototype devices from the sheep subarachnoid space was measured and 8 g of force was the maximum force measured. Some adherent host tissue was observed in 1 of the 6 retrieved prototype devices at explant

reversed the cold allodynia, thermal hyperalgesia, and limb temperature associated with the CCI model. These effects persisted for at least 9 weeks (i.e., the duration of the study) (26).

These pre-clinical rodent models may be designed to test for the possibility that tolerance develops to the chromaffin cell implants. The issue of tolerance to opioids is particularly critical for treating chronic pain and tolerance clearly develops with chronic systemic and local (directly to the spinal cord) administration of opioids in animal models. The issue of tolerance to adrenal cell transplants has added importance because chronic exposure to the catecholamines and opioid peptides secreted by adrenal cell transplants could accelerate tolerance to the analgesic substances produced by the implant or even produce cross-tolerance to exogenous opioids. Fortunately, pre-clinical studies have demonstrated little evidence of tolerance associated with the basal activity of spinal chromaffin cell implants (58) although continuous nicotine administration does seem to produce some degree of analgesic tolerance. Moreover, long-term chromaffin cell implants do not produce cross-tolerance to systemically administered morphine (26).

5.3. Clinical trials using unencapsulated chromaffin cells

The first clinical evaluation of adrenal chromaffin cells implants was conducted by Winnie and associates in 1993 (18, 19). Chromaffin cells obtained from human cadaveric donors were implanted into the subarachnoid space of 5 subjects suffering from intractable pain. Single-donor adrenal glands were prepared for each subject by isolating the medullary tissue and maintained it in tissue culture for approximately one week to ensure viability and functionality. Each patient received cyclosporine prior to implantation to suppress the immune recognition and rejection of the allografts and for 2 weeks after

implantation. Pain scores and analgesic consumption were monitored and CSF samples were taken for determinations of catecholamine and met-enkephalin levels. Four of the five patients exhibited improved pain control within 4 to 6 weeks. Three of these patients demonstrated significant improvements in the reduction of their pain scores and analgesic drug consumption, and reported improved activity. While CSF catecholamine and met-enkephalin levels were elevated over pre-implant levels, these measures were highly variable, and thus only trends could be ascertained, not statistical correlates. Whether this variability was inherent to the donor tissue, the host environment, or some unidentified aspect of the isolation procedure is unclear.

Recently, a report of additional patients from the Lazorthes *et al.* trials has been described in a prospective phase II clinical study (57). This study evaluated 15 patients transplanted intrathecally with adrenal medullary allografts. The inclusion criteria were patients that exhibited intractable cancer pain refractory to systemic opioid treatment due to persistent undesirable side-effects. Prior to implantation, all the patients had their pain controlled by daily intrathecal morphine administration to establish a minimum relief level. Analgesic activity of the adrenal medullary allografts was determined according to the complementary requirement of analgesics, especially with regards to intrathecal morphine intake required to effectively control pain. Five of the 12 patients no longer required intrathecal morphine, two decreased their morphine intake and the remaining five exhibited stable intrathecal morphine intake until the end of their follow-up (average of 4.5 months). In most cases, a relationship between an improved analgesic response and CSF met-enkephalin levels was noted. Although this study provided additional feasibility and safety data to the original series, a large, placebo-controlled trial would be warranted in a larger series of patients with assessment of functional

Clinical Prototype Pain Relieving Devices

status, an extended follow-up period and quality of life.

5.4. Clinical trials using encapsulated chromaffin cells

Although the initial clinical trials of unencapsulated chromaffin cell implants provide compelling proof-of-principle, the widespread use of this approach is unlikely due to the lack of available organ donors, practical limitations of on-site preparation of the cells prior to implantation, and the likely need for immunosuppression to prevent rejection of the implanted chromaffin cell allografts. Xenogenic, encapsulated transplants obtained from a readily available commercial source would be an ideal solution. Moreover, adrenal chromaffin cell transplants for pain management are well suited for immunoisolated cell therapy since they are a nondividing primary cell population and maintain their function and viability in culture and *in vivo* for long periods of time (i.e., up to 500 days post-implantation) (43). In addition, pre-clinical trials with encapsulated bovine adrenal chromaffin cells in a clinically relevant sheep spinal implant site (with respect to similar spinal spaces of implantation) have confirmed sustained viability and catecholamine release under basal and nicotine-stimulated conditions in devices recovered from the intrathecal space of sheep after 4 weeks (42). Similar to what is demonstrated in the present report at 6 weeks, the catecholamine levels from retrieved encapsulated cell devices in the Joseph *et al.* study were comparable to pre-implant levels.

To follow-up on these preliminary encouraging results of both pre-clinical safety studies, as well as the encouraging reports from unencapsulated chromaffin cell allografts, human Phase I clinical trials of encapsulated bovine chromaffin cell implants were conducted in the United States and Switzerland. In these studies, 1-3 x 10⁶ bovine adrenal chromaffin cell preparations were isolated as described in the present study, encapsulated in the device described herein, and were maintained in culture for at least 30 days for sterility and catecholamine release testing. These devices were then inserted into the lumbar subarachnoid space of human volunteers suffering from intractable chronic cancer pain with the minimally surgical procedure described in the present study. Data from these Phase I trials focused primarily on safety issues (30-32). Most of the adverse events observed in trial were directly related to the individual's underlying disease. Of the adverse events related to the cell therapy implants, such as postlumbar puncture headaches, virtually all were self-limited and related to the lumbar puncture performed to insert the device. In summary, these small, open label trials demonstrated that the implantation procedure was minimally invasive and well tolerated. Because reductions in morphine intake were also noted following implantation (suggesting efficacy), larger scale, randomized studies were initiated in a collaborative study between CytoTherapeutics and Astra Pharmaceuticals. Unfortunately, the trials were halted because the efficacy achieved did not reach a level high enough to warrant further study.

5.5. Additional comments and conclusion

There is a relatively extensive list of literature to support the therapeutic potential of encapsulated

chromaffin cells for treating chronic pain. But it is also interesting that several recent reports have indicated that encapsulated adrenal chromaffin cell implants may not produce efficacy as originally demonstrated (59-61). Extensive studies in acute and chronic rodent pain models have failed to find any evidence of analgesia. This lack of effect occurred under conditions that were apparently designed to exactly reproduce previous testing procedures that did demonstrate efficacy. Among the variables examined were the location of implant (intrathecal vs intraventricular), a wide range in cell preparation techniques, and an exhaustive battery of acute and chronic pain tests with and without nicotine stimulation. The authors reported that systemic administration of morphine produced significant analgesia when tested in parallel in the same models. While subtle testing differences cannot be ruled out as contributing factors in the differences between these recent and previous studies, together with the only well controlled clinical trial conducted to date, it appears that, at the least, adrenal chromaffin cells may not produce analgesic effects as consistently as previous reported. Nonetheless, the experience obtained to date has provided an excellent foundation for additional experimentation with primary cells and cell lines (62) designed to secrete factors transplanted into the spinal cord of patients suffering from chronic pain.

Lastly, it is important to keep in mind that pain (acute and chronic) is associated with complex physiological processes. Conceivably, modifying several different transmitter/peptide systems, either alone or in combination, could produce significant functional benefits. Along these lines, intrathecal grafting of numerous cell types including neuroblastoma cells (63) and neurons modified to secrete GABA (64, 65), galanin (66), 5-HT (67), and BDNF (68) have all shown promise in rodent models of pain. The present studies, which demonstrated abundant cell survival secretion profiles in large animals without any detectable untoward side effects, suggests that cell encapsulation may be ideally suited for evaluating long-term delivery of analgesic compounds directly to the spinal cord and may further contribute to understanding the biological processes contributing to the manifestation of chronic pain.

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7. REFERENCES

1. Sladek, J. R. and D. M. Gash: *Neural Transplants*, Eds. J. R. Sladek and D. M. Gash, Plenum, New York (1984)
2. Björklund, A and U. Stenevi: *Neural Grafting in the*

Clinical Prototype Pain Relieving Devices

Mammalian CNS, Eds. Björklund A and U. Stenevi, Elsevier Science, Amsterdam, 709 (1995)

3. Sortwell, C. E., F. Petty, G. Kramer and J. Sagen: *In vivo* release of catecholamines from xenogeneic chromaffin cell grafts with antidepressive activities. *Exp Neurol* 130, 1-8 (1994)

4. Czech, K. and J. Sagen: Update on cellular transplantation into the CNS as a novel therapy for chronic pain. *Prog Neurobiol* 46, 507-522 (1995)

5. Barami, K. and F. G. Diaz: Cellular transplantation and spinal cord injury. *Neurosurg* 47, 691-7000 (2000)

6. Livett, B., D. M. Dean, L. G. Whelan, S. Udenfield and J. Rossier: Co-release of enkephalin and catecholamines from cultured adrenal chromaffin cells. *Science* 225, 734-737 (1981)

7. Yang, H. Y. T., T. Hexum and E. Costa: Opioid peptide in the adrenal gland. *Life Sci* 27, 1119-1125 (1980)

8. Wilson, S., K. Chang and O. H. Viveros: Proportional secretion of opioid peptides and catecholamines from adrenal chromaffin cells in culture. *J Neurosci* 2, 1150-1156 (1982)

9. Carmichael, S. and H. Winkler: The adrenal chromaffin cell. *Sci Amer* 253, 40-49 (1985)

10. Hua, X. Y., J. H. Boublik, M. A. Spicer, J. E. Rivier, M. R. Brown and T. L. Yaksh: The antinociceptive effects of spinally administered neuropeptide Y in the rat: systemic studies on structure-activity relationship. *J Pharmacol Exp Ther* 258, 243-248 (1991)

11. Coupland, R. E: The natural history of the chromaffin cell: Twenty-five years on the beginning. *Arch Histol Cytol* 52, S331-341 (1989)

12. Yaksh, T. L. and S. V. R. Reddy: Studies in the primate on the analgesic effects associated with intrathecal actions of opiates, alpha-adrenergic agonists and baclofen. *Anesthesiology* 54, 451-467 (1981)

13. Sagen, J., G. D. Pappas and M. J. Perlow: Adrenal medullary transplants in the rat spinal cord reduce pain sensitivity. *Brain Res* 384, 189-194 (1986)

14. Sagen, J., G. D. Pappas and H. Pollard: Analgesia induced by isolated bovine chromaffin cells implanted in rat spinal cord. *Proc Natl Acad Sci USA* 83, 7552 (1986)

15. Sagen, J., G. D. Pappas and M. J. Perlow: Alterations in nociception following adrenal medullary transplants into the rat periaqueductal gray. *Exp. Brain Res* 67, 373-379 (1987)

16. Sagen, J., H. Wang and G. D. Pappas: Adrenal medullary tissue implants in rat spinal cord reduce

nociception in a chronic pain model. *Pain* 42, 69-79 (1990)

17. Sagen, J., J. Kemmler and H. Wang: Adrenal medullary transplants increase spinal cord cerebrospinal fluid catecholamine levels and reduce pain sensitivity. *J Neurochem* 56, 623-627 (1991)

18. Sagen, J., G. D. Pappas and A. P. Winnie: Alleviation of pain in cancer patients by adrenal medullary transplants in the spinal subarachnoid space. *Cell Transplant.* 2, 259-266 (1993)

19. Winnie, A., G. D. Pappas, T. K. Gupta, H. Wang, J. Ortega and J. Sagen: Subarachnoid adrenal medullary transplants for terminal cancer pain. *Anesthesiology* 79, 644-653 (1993)

20. Lazorthes, Y., J. C. Bès, J. Sagen, M. Tafani, J. Tkaczuk, B. Sallerin, I. Nahri, J. C. Verdié, E. Ohayon, C. Caratero and G. D. Pappas: Transplantation of human chromaffin cells for intractable cancer pain control. *Acta Neurochir* 64S, 97-100 (1995)

21. Lazorthes, Y., G. D. Pappas, J. C. Bès, J. Sagen, M. Tafani, A. P. Winnie, and J. Tkaczuk: Human chromaffin cell therapy for cancer pain: clinical, biochemical and anatomical data with long-term follow-up. *Acta Neurochir* 138, 606-610 (1996)

22. Ehde, D. M., M. P. Jensen, J. M. Engel, J. A. Turner, A. J. Hoffman and D. D. Cardenas: Chronic pain secondary to disability: a review. *Clin. J Pain* 19, 3-17 (2003)

23. Cancer pain relief and palliative care. Geneva, Switzerland: *World Health Organization*, technical report series, 804, (1990)

24. Ballantyne, J. C: Chronic pain following treatment for cancer: the role of opioids. *The Oncologist* 8, 567-575, (2003)

25. Hama, A. T. and J. Sagen: Reduced pain-related behavior by adrenal medullary transplants in rats with experimental pain neuropathy. *Pain* 52, 223-231 (1993)

26. Hama, A. T. and J. Sagen: Alleviation of neuropathic pain symptoms by xenogeneic chromaffin cell grafts in the spinal subarachnoid space. *Brain Res* 651, 183-193 (1994)

27. Sagen, J., H. Wang, A. T. Hama and G. D. Pappas: Adrenal chromaffin cells: preparation and use in pain models. In: C. Ricordi, Editor, *Methods in Cell Transplantation*, Landes, Austin (1995)

28. Siegan, J. B. and J. Sagen: Attenuation of formalin pain responses in the rat by adrenal medullary transplants in the spinal subarachnoid space. *Pain* 70, 279-285 (1997)

29. Decosterd, I., E. Buchser, N. Gilliard, J. Saydoff, A. D. Zurn and P. Aebischer: Intrathecal implants of bovine chromaffin cells alleviate mechanical allodynia in a rat

Clinical Prototype Pain Relieving Devices

model of neuropathic pain. *Pain* 76, 159-166 (1998)

30. Aebischer, P., E. Buchser, J. M. Joseph, J. Favre, N. de Tribolet, M. Lysaught, S. Rudnick, and M. Goddard: Transplantation in humans of encapsulated xenogeneic cells without immunosuppression: A preliminary report. *Transplantation* 58, 1-3, (1994)

31. Burgess, F. W., M. Goddard, D. Savarese, and H. Wilkinson: Subarachnoid bovine adrenal chromaffin cell implants for cancer pain management. *Anesthesiology* 85, 796 (1996)

32. Buchser, E., M. Goddard, B. Heyd, J. M. Joseph, J. Favre, N. de Tribolet, M. Lysaught, and P. Aebischer: Immunoisolated xenogeneic chromaffin cell therapy for chronic pain: initial clinical experience. *Anesthesiology* 85, 1005-1012 (1996)

33. Sagen, J., H. Wang, P. Tresco and P. Aebischer: Transplants of immunologically isolated xenogeneic chromaffin cells provide a long-term source of pain-reducing neuroactive substances. *J Neurosci* 13, 2415-2423 (1993)

34. Aebischer, P., S. R. Winn and P. M. Galletti: Transplantation of neural tissue in polymer capsules. *Brain Res* 448, 364-368 (1988)

35. Christenson, L., K. E. Dionne and M. J. Lysaught: Biomedical applications of immobilized cells. In: *Fundamentals of Animal Cell Encapsulation and Immobilization*. Eds: M.F.A. Goosen. CRC Press, Inc., Boca Raton, FL, 7-41 (1993)

36. Winn, S. R. and P. A. Tresco: Hydrogel applications for encapsulated cellular transplants. In: *Methods in Neuroscience: Providing Pharmacological Access to the Brain*. Eds: T. R. Flanagan, D. F. Emerich and S. R. Winn. Academic Press, San Diego, CA, Vol. 21, 387-402 (1994)

37. Aebischer, P., P. A. Tresco, J. Sagen and S. R. Winn: Transplantation of microencapsulated bovine chromaffin cells reduces lesion-induced rotational asymmetry in rats. *Brain Res* 560, 43-49 (1991)

38. Crooks, C. A., J. A. Douglas, R. L. Broughton and M. V. Sefton: Microencapsulation of mammalian cells in a HEMA-MMA copolymer: effects on capsule morphology and permeability. *J Biomed Mater Res* 24, 1241-1262 (1990)

39. Gentile, F. T., E. J. Doherty, D. H. Rein, M. S. Shoichet and S. R. Winn: Polymer science for cell encapsulation. *J Reactive Polymers* 25, 207-227 (1995)

40. Shoichet, M. S. and S. R. Winn: Cell delivery to the central nervous system. *Adv Drug Deliv Rev* 42, 81-102, (2000)

41. Emerich, D. F. and S. R. Winn: Immunoisolation cell therapy for CNS Diseases. *Crit Rev Ther Drug*

Carrier Syst 18, 265-298, (2001)

42. Joseph, J. M., M. B. Goddard, J. Mills, V. Padrun, A. Zurn, B. Zielinski, J. Favre, J. P. Gardez, F. Mosimann, J. Sagen, L. Christenson and P. Aebischer: Transplantation of encapsulated bovine chromaffin cells in the sheep subarachnoid space: A preclinical study for the treatment of cancer pain. *Cell Transpl* 5, 355-364 (1994)

43. Lindner, M. D., M. A. Plone, B. Frydel, F. A. Kaplan, P. M. Krueger, W. J. Bell, T. J. Blaney, S. R. Winn, S. S. Sherman, E. J. Doherty and D. F. Emerich: Intraventricular encapsulated calf adrenal chromaffin cells: viable for at least 500 days *in vivo* without detectable adverse effects on behavioral/cognitive function or host immune sensitization in rats. *Restor Neurol Neurosci* 11, 21-35 (1997)

45. Gentile, F. T., E. J. Doherty, D. H. Rein, M. S. Shoichet and S. R. Winn: Polymer science for macroencapsulation of cells for central nervous system transplantation. *Reactive Polymers* 25, 207-227 (1995)

46. Gaya, S. B., A. J. Rees, R. I. Lechler, G. Williams and P. D. Mason: Malignant disease in patients with long-term renal transplants. *Transplantation* 59, 1705-1709 (1995)

47. London, N. J., S. M. Farmery, E. J. Will, A. M. Davison and J. P. Lodge: Risk of neoplasia in renal transplant patients. *Lancet* 346, 403-406 (1995)

48. Balistreri, F., J. C. Bucuvalas and F. C. Ryckman: The effect of immunosuppression on growth and development. *Liver Transpl Surg* 1, 64-73 (1995)

49. Gremlich, S., R. Roduit and B. Thorens: Dexamethasone induces posttranslational degradation of GLUT2 and inhibition of insulin secretion in isolated pancreatic beta cells. Comparison with the effects of fatty acids. *J Biol Chem* 272, 3216-3222 (1997)

50. Hahn, H. J., F. Laube, S. Kloting, K. D. Kohnert and R. Warzock: Toxic effects of cyclosporin on the endocrine pancreas of Wistar rats. *Transplantation* 41, 44-47 (1986)

51. Aebischer, P., P. A. Tresco, S. R. Winn, L. A. Greene and C. B. Jaeger: Long-term cross-species brain transplantation of a polymer encapsulated dopamine-secreting cell line. *Exp Neurol* 111, 269-275 (1991)

52. Tresco, P. A., S. R. Winn, C. B. Jaeger, L. A. Greene and P. Aebischer: Polymer-encapsulated PC12 cells: long-term survival and associated reduction in lesion-induced rotational behavior. *Cell Transpl* 1, 255-264 (1992)

53. Sagen, J., A. T. Hama, S. R. Winn, P. A. Tresco and P. Aebischer: Pain reduction by spinal implantation of xenogeneic chromaffin cells immunologically-isolated in polymer capsules. *Soc Neurosci Abstr* 19, 234 (1993)

54. Winn, S. R., M. D. Lindner, G. Hagggett, J. M. Francis and D. F. Emerich: Polymer-encapsulated genetically-modified cells continue to secrete human nerve growth

Clinical Prototype Pain Relieving Devices

factor for over one year in rat ventricles: behavioral and anatomical consequences. *Exp Neurol* 140, 126-138 (1996)

55. Subramanian, T., D. F. Emerich, R. A. E. Bakay, J. M. Hoffman, M. M. Goodman, T. M. Shoup, G. W. Miller, A. I. Levey, G. W. Hubert, S. Batchelor, S. R. Winn, J. A. Saydoff and R. L. Watts: Polymer-encapsulated PC12 cells demonstrate high affinity uptake of dopamine *in vitro* and 18F-dopa uptake and metabolism after intracerebral implantation in nonhuman primate. *Cell Transpl* 6, 469-477 (1997)

56. Aebischer, P., M. Schlep, N. Deglon, J-M. Joseph, L. Hirt, B. Heyd, M. Goddard, J. P. Hammang, A. D. Zurn, A. C. Kato, F. Regli and E. E. Baetge: Intrathecal delivery of CNTF using encapsulated genetically modified xenogeneic cells in amyotrophic lateral sclerosis patients. *Nature Med* 2, 696-699 (1996)

57. Date, T. Shingo, T. Ohmoto and D. F. Emerich: Long-term enhanced chromaffin cell survival and behavioral recovery in hemiparkinsonian rats with co-grafted polymer-encapsulated human NGF-secreting cells. *Exp Neurol* 147, 10-17 (1997)

58. Lazorthes, Y., J. Sagen, B. Sallerin, J. Tkaczuk, H. Duplan, J. C. Sol, M. Tafani, and J. C. Bès: Human chromaffin cell graft into the CSF for cancer pain management: a prospective phase II clinical study. *Pain* 87, 19-32 (2000)

59. Wang, H. and J. Sagen: Absence of appreciable tolerance and morphine cross-tolerance in rats with adrenal medullary transplants in the spinal cord. *Neuropharmacology* 33, 681-692 (1994)

60. Lindner, M. D., J. M. Francis, M. A. Plone, P. E. McDermott, B. R. Frydel, D. F. Emerich and J. A. Saydoff: The analgesic potential of intraventricular polymer-encapsulated adrenal chromaffin cells in a rodent model of chronic neuropathic pain. *Exp Clin Psychopharmacol* 8, 524-538 (2000)

61. Lindner, M. D., J. M. Francis and J. A. Saydoff: Intrathecal polymer-encapsulated bovine chromaffin cells fail to produce analgesic effects in the hotplate and formalin tests. *Exp Neurol* 165, 370-383 (2000)

62. Lindner, M. D., J. M. Francis, P. E. McDermott, W. J. Bell, T. J. Blaney, S. S. Sherman and J. A. Saydoff: Numerous adrenal chromaffin cell preparations fail to produce analgesic effects in the formalin test or in tests of acute pain even with nicotine stimulation. *Pain* 88, 177-188 (2000)

63. Eaton, M. J., B. R. Frydel, T. L. Lopez, X. T. Nie, J. Huang, J. McKillop and J. Sagen: Generation and initial characterization of conditionally immortalized chromaffin cells. *J Cell Biochem* 79, 38-57 (2000)

64. De la Calle, J. L., M. A. Mena, J. R. Gonzalez-Escalada and C. L. Paino: Intrathecal transplantation of

neuroblastoma cells decreases heat hyperalgesia and cold allodynia in a rat model of neuropathic pain. *Brain Res Bull* 59, 205-11 (2002)

65. Eaton, M. J., J. A. Plunkett, M. A. Martinez, T. Lopez, S. Karmally, P. Cejas and S. R. Whittemore: Transplants of neuronal cells bioengineered to synthesize GABA alleviate chronic neuropathic pain. *Cell Transpl* 8, 87-101 (1999)

66. Stublely, L. A., M. A. Martinez, S. Karmally, T. Lopez, P. Cejas and M. J. Eaton: Only early intervention with gamma-aminobutyric acid cell therapy is able to reverse neuropathic pain after partial nerve injury. *J Neurotrauma* 18, 471-7 (2001)

67. Eaton, M. J., S. Karmally, M. A. Martinez, J. A. Plunkett, T. Lopez and P. J. Cejas: Lumbar transplant of neurons genetically modified to secrete galanin reverse pain-like behaviors after partial sciatic nerve injury. *J Peripher Nerv Syst* 4, 245-57 (2000)

68. Eaton, M. J., D. I. Santiago, H. A. Dancausse and S. R. Whittemore: Lumbar transplants of immortalized serotonergic neurons alleviate chronic neuropathic pain. *Pain* 72, 59-69 (1997)

69. Cejas, P. J., M. Martinez, S. Karmally, M. McKillop, J. McKillop, J. A. Plunkett, M. Oudega and M. J. Eaton: Lumbar transplant of neurons genetically modified to secrete brain-derived neurotrophic factor attenuates allodynia and hyperalgesia after sciatic nerve constriction. *Pain* 86, 195-210 (2000)

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